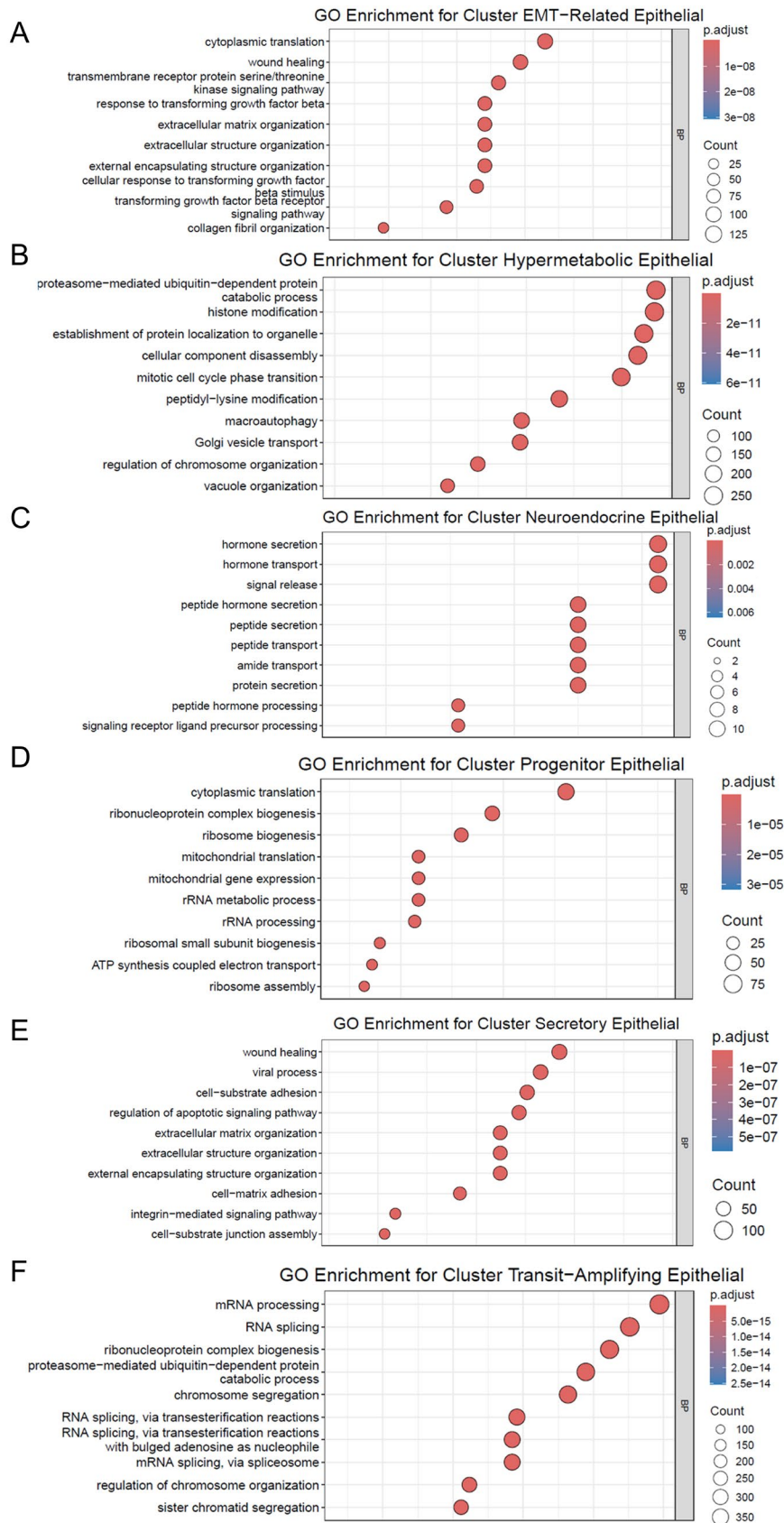
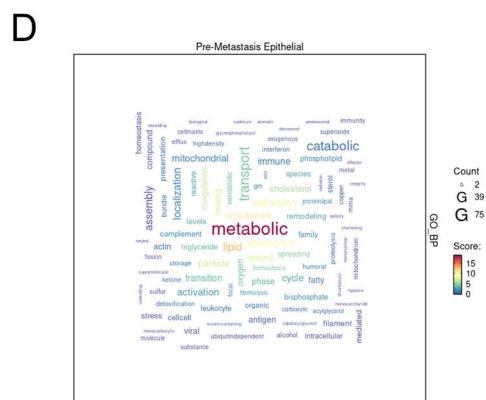
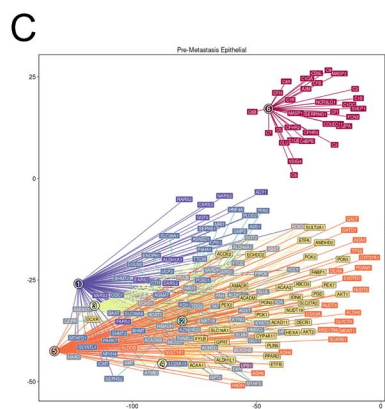
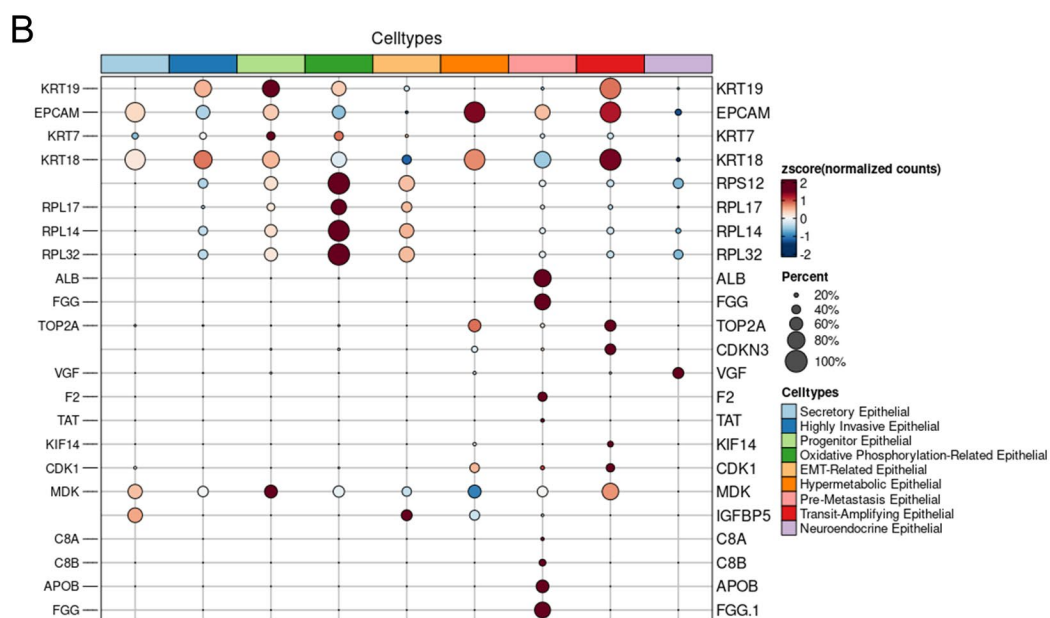
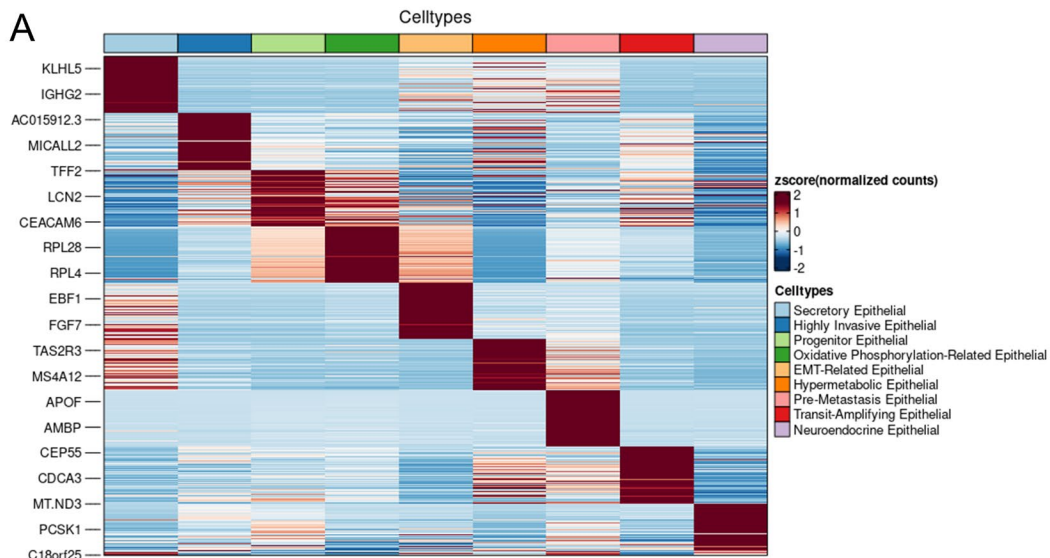




Supplementary Figure 1 Gene Ontology (GO) enrichment analysis of epithelial cell subtypes

(A–F) Bubble plots displaying GO functional enrichment analysis of genes specifically upregulated in epithelial cell subtypes, including EMT-related epithelial (A), hypermetabolic epithelial (B), neuroendocrine epithelial (C), progenitor epithelial (D), secretory epithelial (E), and transit-amplifying epithelial (F). Each plot shows the top 10 significantly enriched biological processes. The horizontal axis indicates gene counts, while the vertical axis indicates enriched GO biological processes. Bubble size represents the number of genes enriched in each biological process, and bubble color represents the adjusted significance level (p.adjust), with darker colors indicating higher enrichment significance.

Specifically, the EMT-related epithelial subset (A) was enriched in extracellular matrix remodeling, wound healing, and response to transforming growth factor-beta (TGF- β). The hypermetabolic subset (B) showed significant enrichment in protein ubiquitination, autophagy, and organelle catabolism pathways. The neuroendocrine subset (C) was predominantly enriched in hormone secretion and signaling. The progenitor epithelial subset (D) displayed enrichment in ribosome biogenesis, mitochondrial function, and energy metabolism. The secretory subset (E) was enriched in response to viral processes, wound healing, and cell matrix adhesion. The transit-amplifying subset (F) was enriched in mRNA processing, RNA splicing, and chromosome segregation, closely associated with cell cycle and proliferation. These results highlight the distinctive biological functions and regulatory pathways associated with each epithelial cell subset, demonstrating functional heterogeneity within the epithelial compartment of the tumor microenvironment.



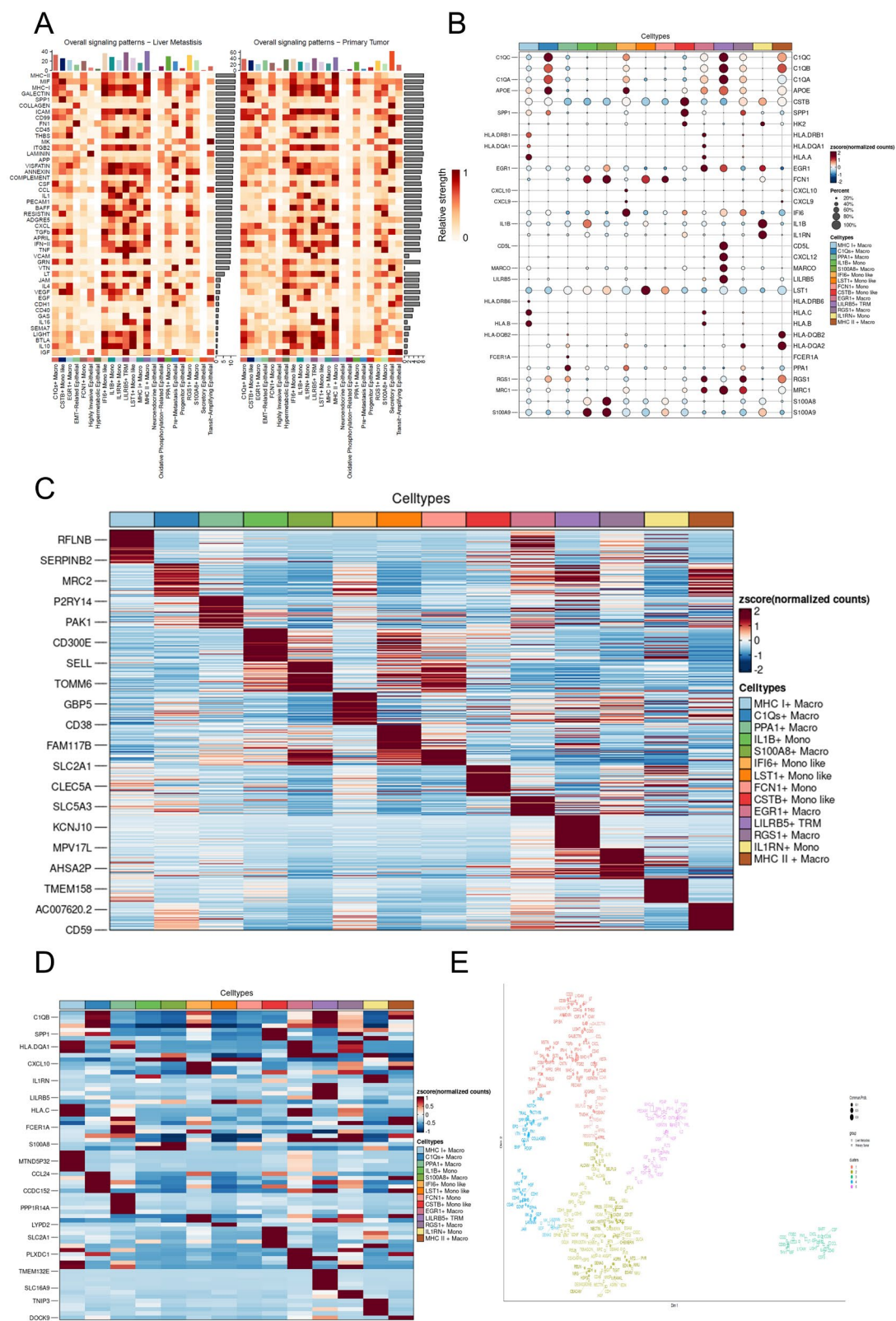
Supplementary Figure 2. Marker-based characterization and functional annotation of epithelial subtypes.

(A) Heatmap showing scaled expression of representative marker genes across the annotated epithelial subtypes, including secretory epithelial, highly invasive epithelial, progenitor epithelial, oxidative phosphorylation-related epithelial, EMT-related epithelial, hypermetabolic epithelial, pre-metastasis epithelial, transit-amplifying epithelial, and neuroendocrine epithelial states. Each column represents an epithelial subtype, and rows represent marker genes. The color scale indicates z-score-normalized expression levels.

(B) Dot plot showing the expression of selected canonical and subtype-enriched epithelial markers across epithelial subtypes. Dot size represents the percentage of cells expressing each gene, and color indicates the average z-score-normalized expression level. Markers include broad epithelial genes such as KRT19, EPCAM, KRT7, and KRT18, ribosomal and proliferative markers, hepatocyte-associated genes such as ALB, FGA/FGG, and APOB, and lineage-associated markers including VGF, TAT, KIF14, CDK1, MDK, C8A, and C8B.

(C) Gene Ontology enrichment network of genes enriched in the pre-metastasis epithelial state. Nodes represent enriched biological processes, and edges indicate shared genes between terms. Enriched terms include amino acid metabolic processes, organic acid catabolic processes, carboxylic acid catabolic processes, small-molecule catabolic processes, and complement activation-related processes.

(D) Word cloud summarizing representative GO biological processes enriched in the pre-metastasis epithelial state. Word size reflects the relative prominence of enriched functional categories, highlighting metabolic, catabolic, complement-associated, transport, and immune-related programs.



Supplementary Figure 3. Macrophage/monocyte transcriptional states and differential signaling patterns between liver metastases and primary tumors.

(A) Heatmap comparing overall intercellular signaling patterns inferred by CellChat between liver metastases and primary tumors. Rows represent signaling pathways, and columns represent annotated cell populations. Color intensity indicates relative signaling strength. Liver metastases showed enhanced immune-related signaling patterns, including chemokine, antigen-presentation, and inflammatory pathways, whereas primary tumors displayed relatively stronger growth factor- and matrix-associated signaling programs.

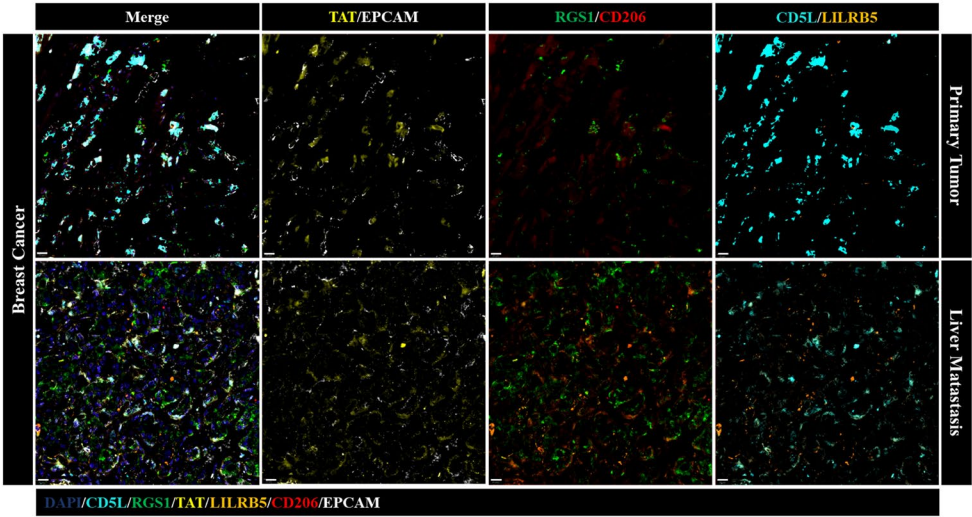
(B) Dot plot showing expression of representative marker genes across macrophage/monocyte subtypes. Dot size represents the percentage of cells expressing each gene, and color indicates the average z-score-normalized expression level. Marker genes include complement-associated genes (C1QA, C1QB, C1QC), antigen-presentation genes (HLA-DRA, HLA-DRB1, HLA-DQA1, HLA-DQA2), inflammatory and chemokine genes (CXCL9, CXCL10, IL1B, CCL5, CXCL12), and subtype-enriched genes such as SPP1, HK2, FCN1, LILRB5, LST1, MARCO, RGS1, MRC1, S100A8, and S100A9.

(C) Heatmap displaying scaled expression of differentially enriched genes across annotated macrophage/monocyte states, including MHC I-high macrophages, C1Q-positive macrophages, PPA1-positive macrophages, IL1B-positive monocytes, S100A8-positive macrophages, IFI6-positive monocyte-like cells, LST1-positive monocyte-like cells, FCN1-positive monocytes, CSTB-positive monocyte-like cells, EGR1-positive macrophages, LILRB5-positive tissue-resident-like macrophages, RGS1-positive macrophages, IL1RN-positive monocytes, and MHC II-high macrophages. The color scale indicates z-score-normalized expression levels.

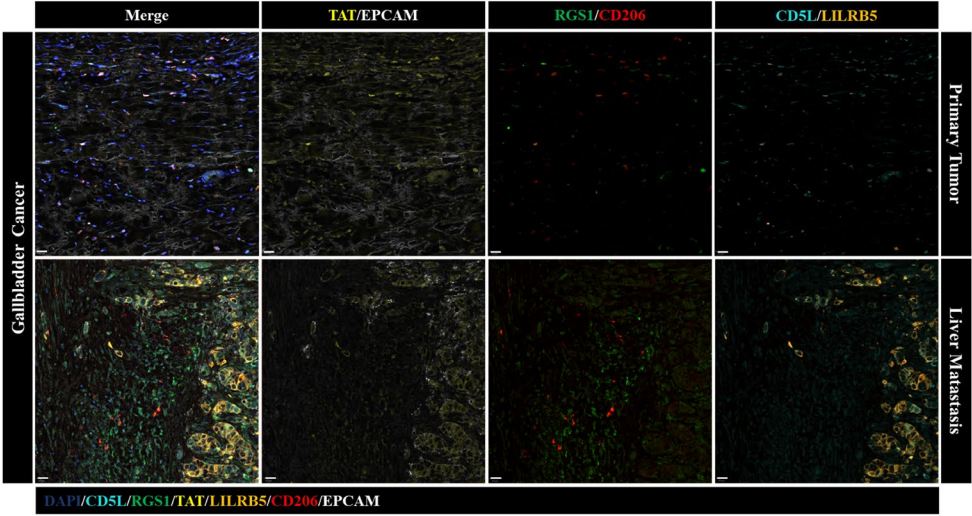
(D) Heatmap of selected marker genes across macrophage/monocyte subtypes, highlighting transcriptional programs associated with complement activation, antigen presentation, inflammatory chemokine signaling, tissue remodeling, and immunoregulatory macrophage states.

(E) Pseudotime trajectory visualization of macrophage/monocyte states. Cells are colored by annotated macrophage/monocyte subtype, and point size indicates relative pseudotime. The trajectory illustrates a transcriptional continuum among inflammatory monocyte-like states, RGS1-high macrophages, and LILRB5-high resident-like macrophage states. These patterns should be interpreted as inferred cell-state ordering rather than definitive lineage relationships.

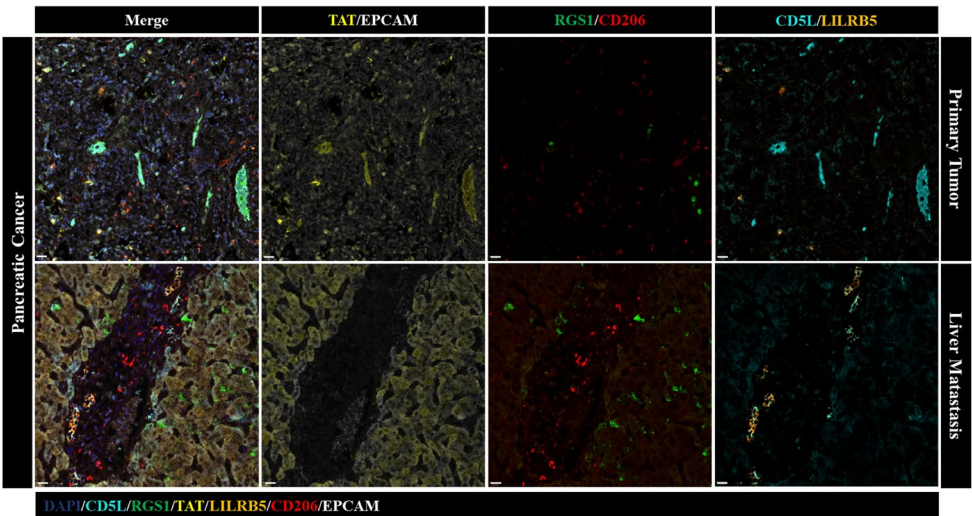
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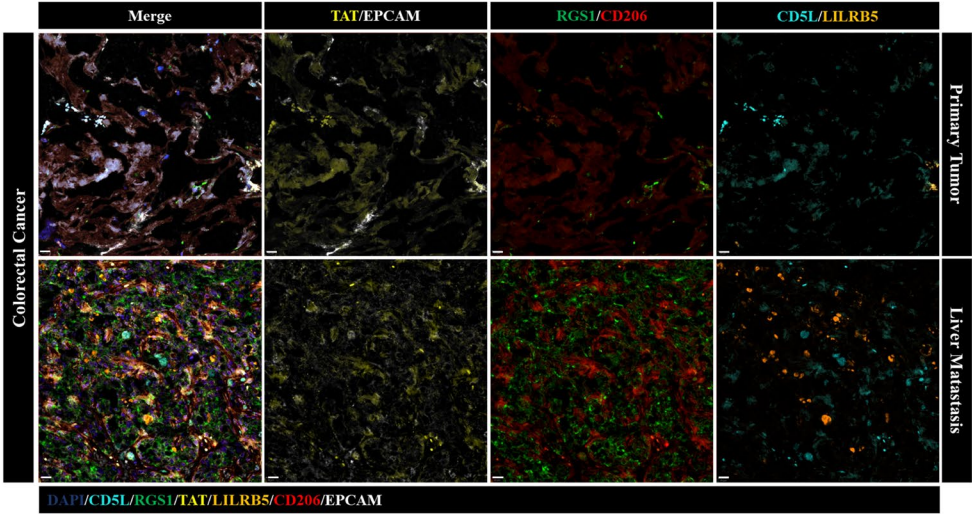
Supplementary Figure 4 Spatial validation of tumor cells and macrophage subtypes in primary tumors and liver metastasis across multiple cancer types

(A–C) Multiplex immunofluorescence staining illustrating spatial distributions and colocalization of liver-adaptive tumor cells (TAT/EPCAM) and macrophage subtypes (RGS1/CD206, CD5L/LILRB5) in breast cancer (A), gallbladder cancer (B), and pancreatic cancer (C). For each cancer type, representative images from primary tumors (upper panels) and matched liver metastasis (lower panels) are shown.

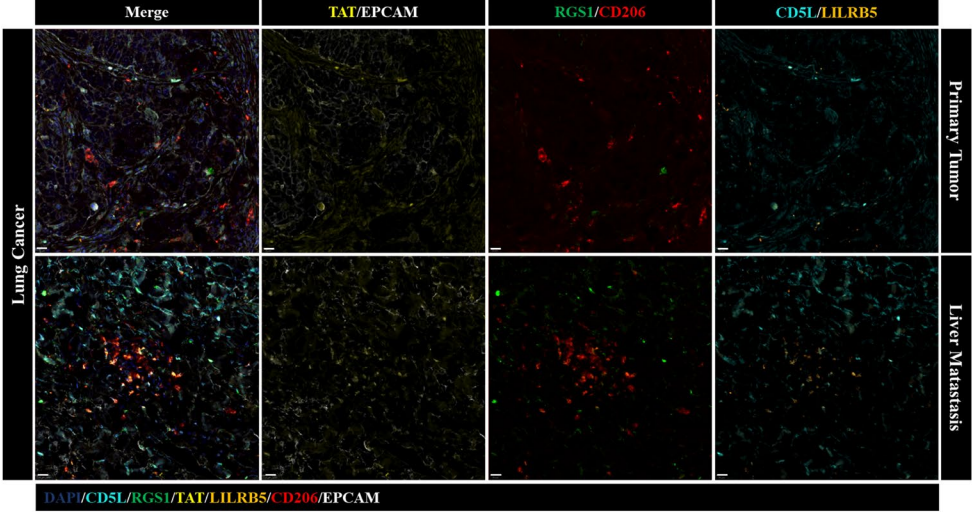
From left to right: merged fluorescence images (Merge); liver-adaptive tumor cells (TAT/EPCAM, yellow and gray); macrophage subtypes co-stained for RGS1 (green) and CD206 (red); and macrophage subtypes co-stained for CD5L (cyan) and LILRB5 (orange). Cell nuclei were stained with DAPI (blue). Scale bar, 20 μm .

Results indicate that liver-adaptive tumor cells exhibited clear spatial colocalization with macrophage subtypes (RGS1+ macrophages, LILRB5+ macrophages, and CD206+ immunoregulatory macrophages) specifically in liver metastasis, while such colocalization was weak or absent in primary tumors. These consistent patterns across multiple cancers further validate a mechanism where tumor cells adapt to the hepatic microenvironment by recruiting and reshaping macrophage subtypes.

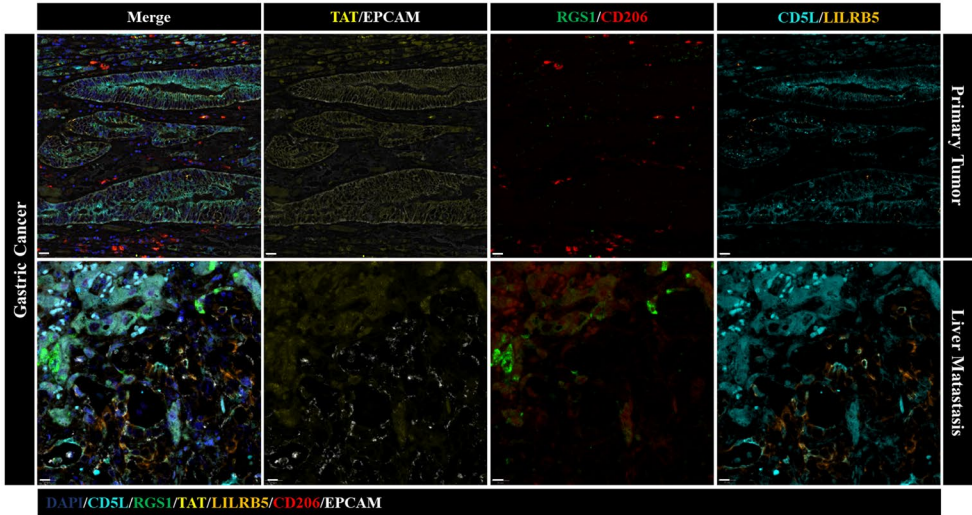
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Supplementary Figure 5 Spatial validation of liver-adaptive tumor cells and macrophage subtypes in colorectal, lung, and gastric cancers

(A–C) Multiplex immunofluorescence staining illustrating spatial distributions and colocalization between liver-adaptive tumor cells (TAT/EPCAM) and macrophage subtypes (RGS1/CD206, CD5L/LILRB5) in colorectal cancer (A), lung cancer (B), and gastric cancer (C). Representative images from primary tumors (top panels) and matched liver metastasis (bottom panels) are shown for each cancer type.

From left to right: merged fluorescence images (Merge); liver-adaptive tumor cells labeled by TAT/EPCAM (yellow and gray); macrophage subtypes co-stained with RGS1 (green) and CD206 (red); and macrophage subtypes co-stained with CD5L (cyan) and LILRB5 (orange). Cell nuclei are stained with DAPI (blue). Scale bars, 20 μm .

Results reveal marked spatial colocalization between liver-adaptive tumor cells and macrophage subtypes, particularly RGS1⁺ and LILRB5⁺ macrophages, in liver metastasis. Such spatial relationships were weak or absent in primary tumors. These consistent findings across cancer types support a stable interaction between tumor cells and macrophages, highlighting the potential role of chemokine-driven recruitment and interaction with macrophage subtypes in establishing a hepatic metastatic niche.

***Supplementary Figure 6* Cell-cell communication network analysis between macrophage/monocyte and T cell subtypes in liver metastasis and primary tumors**

(A) Heatmap comparing overall signaling pathway activities involving macrophage/monocyte subtypes and T cell subtypes in liver metastasis (left) and primary tumors (right). The vertical axis indicates cell communication signaling pathways, while the horizontal axis represents different cell subtypes. Color intensity shows relative signaling strength, with darker colors representing higher pathway activity. Bar plots on the right summarize overall signaling strength per pathway.

(B) 2D clustering plot displaying differentially enriched ligand-receptor pairs between liver metastasis (circles) and primary tumors (squares). Each point represents a ligand-receptor pair, colored according to distinct molecular clusters. Circles and squares indicate pairs specifically enriched in liver metastasis or primary tumors, respectively, with size proportional to communication probability.

These data highlight notable differences in macrophage/monocyte–T cell interactions between liver metastasis and primary tumors. Specifically, liver metastasis shows increased inflammatory signaling, immune regulatory pathways, and chemokine-mediated interactions (e.g., MHC-I, CCL, CXCL, IL16 pathways), whereas primary tumors predominantly feature cell adhesion and baseline immune regulation pathways. These differences reflect the selective remodeling of macrophage–T cell communication during tumor metastasis to the liver.

A



B



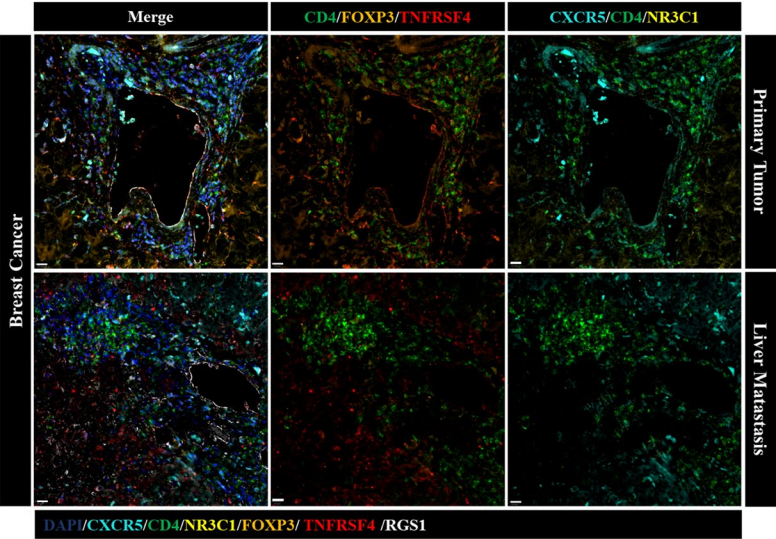
Supplementary Figure 7 Differential gene expression and functional enrichment analysis of pseudotime trajectories for CD4+ and CD8+ T cell subtypes in liver metastasis

(A–B) Monocle pseudotime trajectory analysis demonstrating dynamic gene expression patterns in CD4+ T cells (A) and CD8+ T cells (B) from liver metastasis. Heatmaps illustrate genes significantly changing expression along pseudotime (red: high expression, blue: low expression). Expression trends are shown on the left, with GO enrichment analyses of corresponding gene clusters on the right.

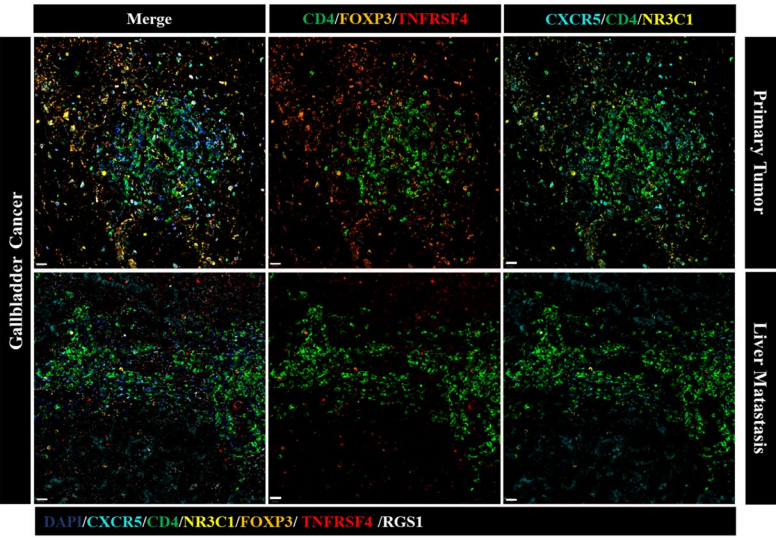
(A) CD4+ T cell pseudotime analysis, initiating from naive lymphocytes and branching towards NR3C1+ follicular helper T cells (NR3C1+ CD4+ Tfh) and TNFRSF4+ regulatory T cells (TNFRSF4+ Treg). Gene enrichment analysis revealed dynamic changes involving T cell activation, immune signaling, and metabolic processes.

(B) CD8+ T cell pseudotime trajectory involving ATP5E+ CD8+ T, GZMK+ NK-like CD8+ T, CCL5+ CD8+ Tem, and GZMB+ NK-like CD8+ Tex subtypes. Differential expression analysis highlighted significant functional shifts in immune effector activity (cytotoxicity and T cell activation), protein translation, stress responses, and RNA processing along the trajectory.

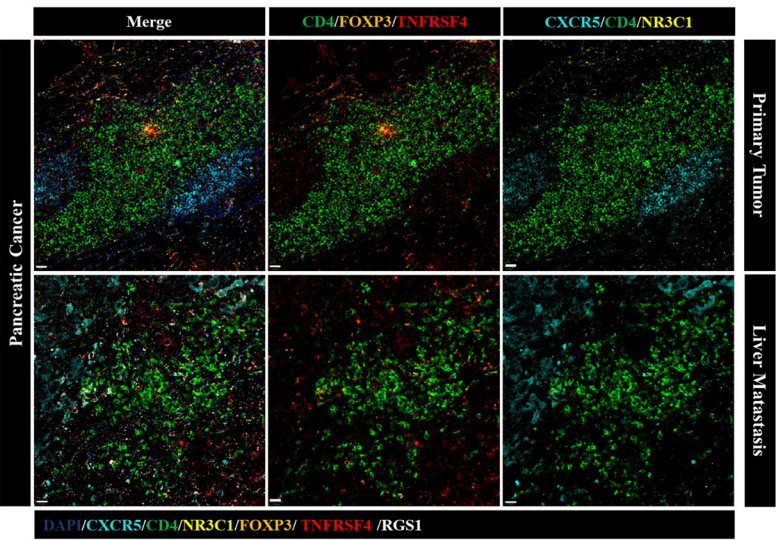
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***Supplementary Figure 8* Multiplex immunofluorescence analysis of spatial localization between CD4⁺ T cell subtypes and RGS1⁺ macrophages in primary tumors and liver metastasis of breast, gallbladder, and pancreatic cancers**

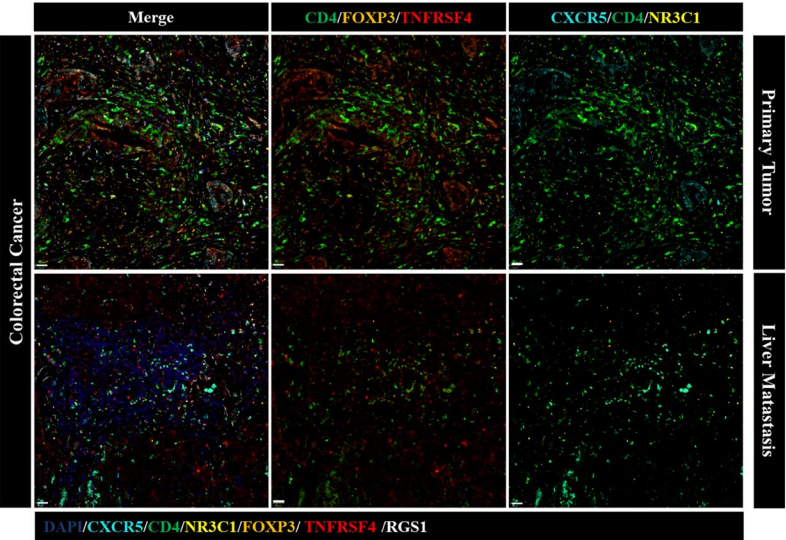
(A–C) Representative multiplex immunofluorescence images showing spatial distribution and colocalization of CD4⁺ T cell subtypes (NR3C1⁺ CD4⁺ Tfh and TNFRSF4⁺ Treg) with RGS1⁺ macrophages in breast cancer (A), gallbladder cancer (B), and pancreatic cancer (C). For each cancer type, images from primary tumors (upper panels) and matched liver metastasis (lower panels) are shown.

From left to right: merged fluorescence images (Merge); co-staining for CD4 (green)/FOXP3 (yellow) and TNFRSF4 (red); co-staining for CXCR5 (cyan)/CD4 (green) and NR3C1 (yellow); and single staining for RGS1⁺ macrophages (white). Cell nuclei are stained with DAPI (blue).

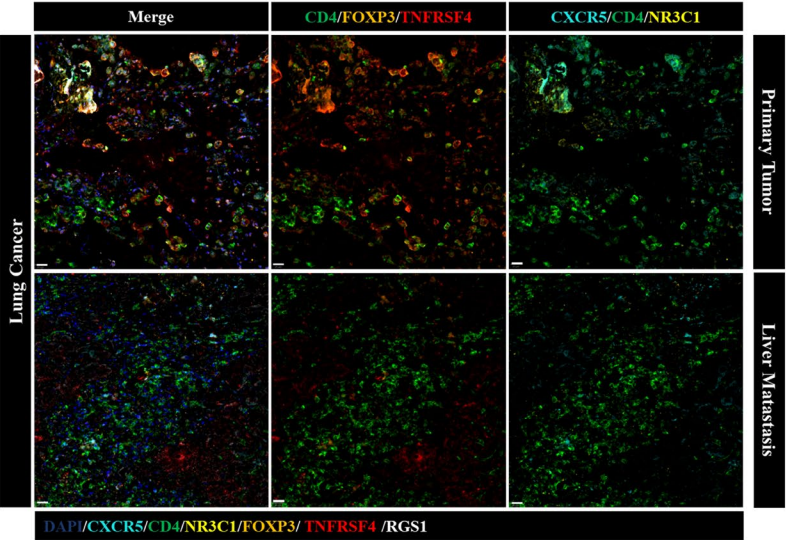
Scale bars, 20 μ m.

Compared to primary tumors, liver metastasis exhibits significant spatial colocalization between NR3C1⁺ CD4⁺ Tfh/TNFRSF4⁺ Treg subtypes and RGS1⁺ macrophages, suggesting potential functional interactions between these immune cell subtypes in the liver metastatic microenvironment. This consistent pattern across cancer types further highlights the crucial role of RGS1⁺ macrophages in immune remodeling during liver metastasis.

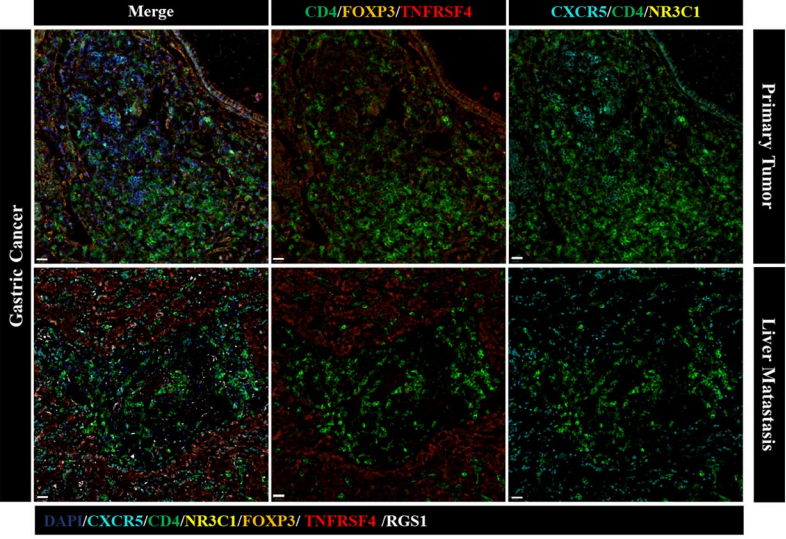
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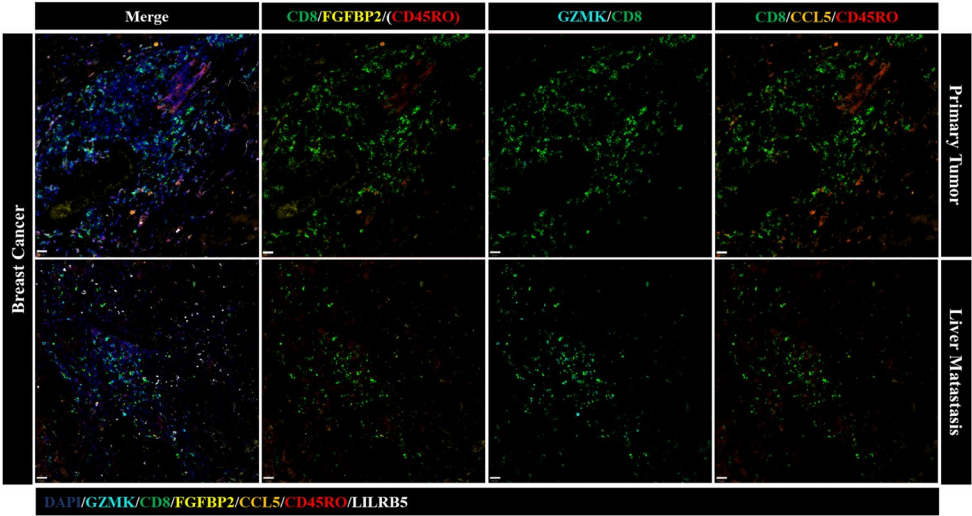
***Supplementary Figure 9* Multiplex immunofluorescence analysis demonstrating spatial localization of CD4⁺ T cell subtypes and RGS1⁺ macrophages in primary tumors and liver metastasis of colorectal, lung, and gastric cancers**

(A–C) Representative multiplex immunofluorescence staining images showing spatial distribution and colocalization patterns of CD4⁺ T cell subtypes (NR3C1⁺ CD4⁺ Tfh and TNFRSF4⁺ Treg) with RGS1⁺ macrophages in colorectal cancer (A), lung cancer (B), and gastric cancer (C). Each cancer type is represented by primary tumor (top panels) and matched liver metastasis tissues (bottom panels).

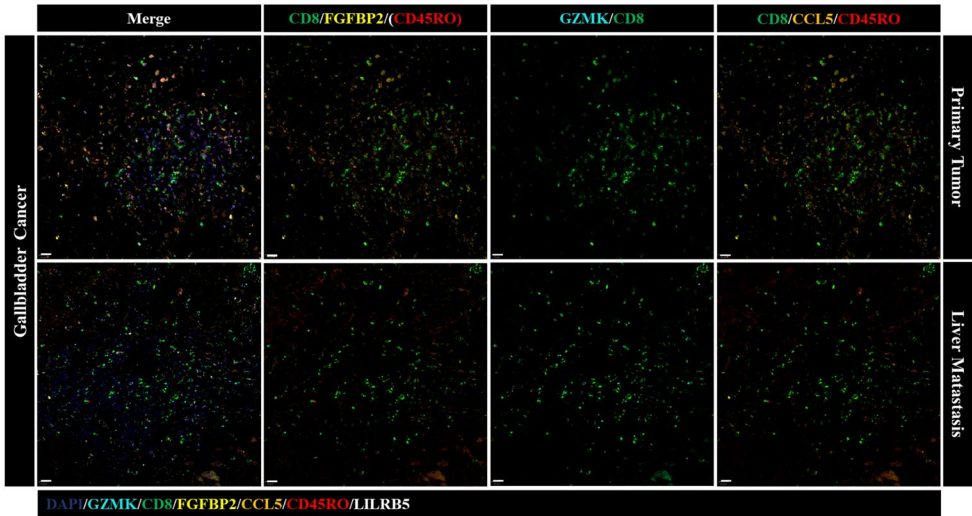
From left to right: merged fluorescence images (Merge); co-staining for CD4 (green)/FOXP3 (yellow) and TNFRSF4 (red); co-staining for CXCR5 (cyan)/CD4 (green) and NR3C1 (yellow); and single staining for RGS1⁺ macrophages (white). Cell nuclei are stained with DAPI (blue). Scale bars, 20 μ m.

Compared to primary tumors, CD4⁺ T cell subtypes (NR3C1⁺ CD4⁺ Tfh and TNFRSF4⁺ Treg) and RGS1⁺ macrophages show enhanced spatial colocalization in liver metastasis, indicating potential cooperative interactions within the liver metastatic microenvironment. This spatial pattern, consistent across cancer types, underscores the critical role of CD4⁺ T cell macrophage interactions in shaping the metastatic immune niche.

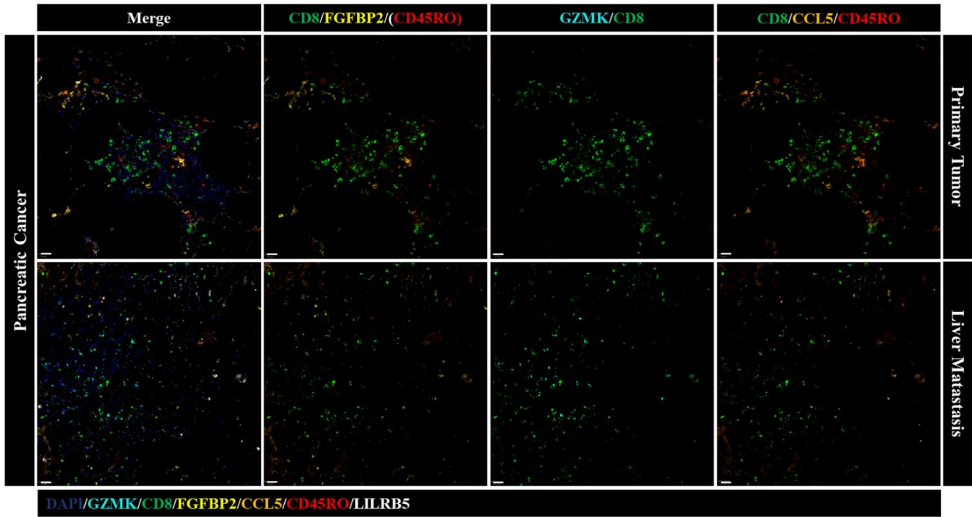
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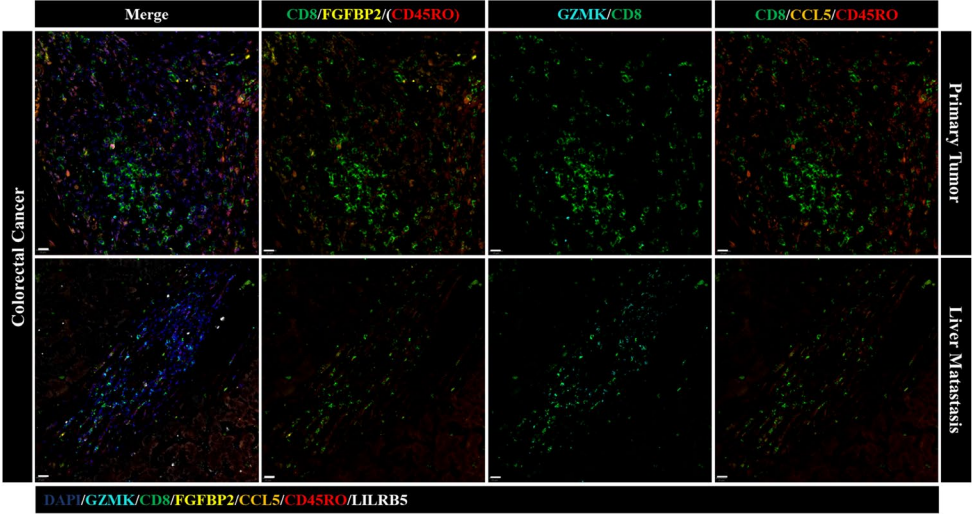
***Supplementary Figure 10* Multiplex immunofluorescence analysis of spatial localization between CD8+ T cell subtypes and LILRB5+ macrophages in primary tumors and liver metastasis of breast, gallbladder, and pancreatic cancers**

(A–C) Representative multiplex immunofluorescence staining images showing spatial distributions and colocalization of CD8+ T cell subtypes (FGFBP2+ CD8+ Temra, GZMK+ NK like CD8+ T, and CCL5+ CD8+ Tem) with LILRB5+ macrophages in breast cancer (A), gallbladder cancer (B), and pancreatic cancer (C). Each cancer type includes primary tumors (top panels) and matched liver metastasis (bottom panels).

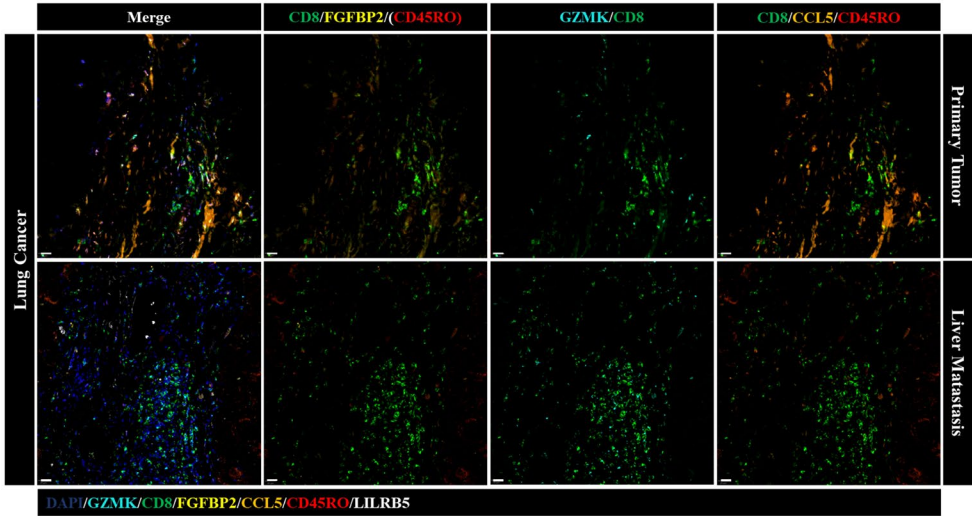
From left to right: merged fluorescence images (Merge); co-staining of CD8 (green) with FGFBP2 (yellow)/CD45RO (red); co-staining of GZMK (cyan) with CD8 (green); and co-staining of CD8 (green) with CCL5 (yellow)/CD45RO (red). LILRB5+ macrophages are separately shown in white. Cell nuclei are stained with DAPI (blue). Scale bars, 20 μ m.

Compared to primary tumors, liver metastasis exhibits notable spatial colocalization and aggregation of CD8+ T cell subtypes and LILRB5+ macrophages, suggesting potential functional interactions. The consistency of this colocalization across cancer types underscores the significance of CD8+ T cell–macrophage interactions within the metastatic liver immune microenvironment.

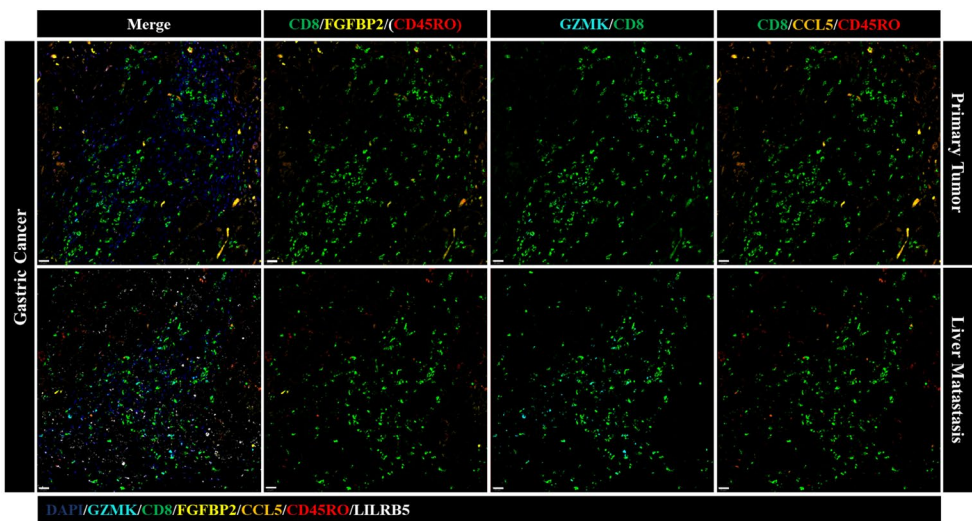
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***Supplementary Figure 10* Multiplex immunofluorescence analysis of spatial localization between CD8⁺ T cell subtypes and LILRB5⁺ macrophages in primary tumors and liver metastasis of colorectal, lung, and gastric cancers**

(A–C) Representative multiplex immunofluorescence staining images showing spatial distribution and colocalization patterns of CD8⁺ T cell subtypes (FGFBP2⁺ CD8⁺ Temra, GZMK⁺ NK-like CD8⁺ T, and CCL5⁺ CD8⁺ Tem) with LILRB5⁺ macrophages in colorectal cancer (A), lung cancer (B), and gastric cancer (C). For each cancer type, images from primary tumors (top panels) and matched liver metastasis (bottom panels) are shown.

From left to right: merged fluorescence images (Merge); co-staining for CD8 (green) with FGFBP2 (yellow)/CD45RO (red); co-staining for GZMK (cyan) with CD8 (green); and co-staining for CD8 (green) with CCL5 (yellow)/CD45RO (red). LILRB5⁺ macrophages are separately displayed in white. Cell nuclei are stained with DAPI (blue). Scale bars, 20 μ m.

Compared to primary tumors, liver metastasis shows clear spatial colocalization and aggregation of CD8⁺ T cell subtypes with LILRB5⁺ macrophages, suggesting potential functional interactions between these immune populations specifically within the liver metastatic niche. The consistency of this colocalization pattern across cancer types further supports the importance of macrophage–CD8⁺ T cell interactions in shaping the metastatic immune microenvironment.